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Lipid Peroxidation and Antioxidant Enzymes in Turkish Population: Relation to Age, Gender, Exercise, and Smoking

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ÖZBAY, B. and DÜLGER, H. *Lipid Peroxidation and Antioxidant Enzymes in Turkish Population: Relation to Age, Gender, Exercise, and Smoking.* Tohoku J. Exp. Med., 2002, **197** (2), 119-124 —— The purpose of this study was to examine the change in lipid peroxidation and antioxidant enzyme activities in healthy subjects and to evaluate the concentrations of superoxide dismutase, glutathione peroxidase and malondialdehyde, an end product of lipid peroxidation in exercise and smoking. Study included 257 appearently healthy individuals, 133 males and 124 females. In all subjects, malondialdehyde (MDA) levels were analyzed as an indicator of the lipid peroxidation activities. Superoxide dismutase, glutathione peroxidase activities were measured as an indicator of antioxidant activities. Oxidative stress was estimated by the method based on thiobarbituric acid reactivity. Erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were estimated on hemolysates by use of commercial available kits (Randox lab., Dublin, Ireland). For all groups serum lipid peroxidation and erythrocyte SOD and GSH-Px were obtained at the initial and the following periods. Serum MDA level was higher in the elderly than in the children and in the adults. MDA levels were higher in the smoking, acute exercise than their counterparts in the control groups. GSH-Px activity was significantly lower in the acute exercise group, and higher in the trained group than those as controls. SOD decreased in the elderly, smoking and acute exercise groups and increased in trained individuals. There was a significant increase in lipid peroxidation activity and a significant decrease in antioxidant enzyme activity in cases of acute exercise and smoking as well as the elderly ———— lipid peroxidation; antioxidants; smoking; exercise

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All of major classes of biomolecules may be attacked by free radical species but lipids are the most susceptible. Cell membranes are rich sources of polyunsaturated fatty acids (PUFA), which are readily attacked by oxidizing radicals. The exudative destruction of PUFA by deleterious free radical reactions, known as lipid peroxidation (Cheeseman 1993). Lipid peroxidation is of particular significance as a damaging reaction for cell components. Than lipid peroxidation has been implicated in a wide range of cell and tissue damages, diseases, biological variables and life habits (Esterbauer and Cheeseman 1987; Cheeseman 1993). The methods most commonly used to detect lipid peroxidation are the measurement of MDA in the blood and tissues (Cheeseman and Slater 1993).

Substances that neutralize the potential degenerative effect of radical-induced lipid peroxidation are generally grouped in the so called antioxidant components, radical scavencers, chain terminators, or reductants (Pal 1994). The antioxidants are responsible for cellular protection against oxidative stress. These scavengers are strategically compartmentalized in subcellular organelles within the cell to provide maximum protection. For instance, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase are not only distributed in the cytosol but also localised in mitochondria (Ji et al. 1988).

SOD exists in virtually all aerobic organisms, and its major function is to catalyse the dismutative reaction. GSH-Px, intracellulary located in the cytosol and mitochondrial matrix of cells, and catalyses the reduction of hydrogen peroxide and detoxificates radical species (Pal 1994). Studies have also assessed changes in the body's natural antioxidants such as glutathion peroxidase, superoxide dismutase. Enzyme activities of GSH-Px, SOD and others (catalase, glutathione reductase) have been used to assess changes in antioxidant status (Clarkson 1995).

In recent years, much attention has been paid to the role radicals and antioxidants play in diseases, aging, biological variables, life habits such as physical activity, tobacco smoking, and living in the cities that have polluted air. Determination of serum malondialdehyde (MDA) levels was chosen to show the degree of oxidative stress, and determinations of erythrocytes SOD and GSH-Px levels to show that how oxidative stress effected erythrocytes. Consequently, our aim was to determine the levels of MDA and endogenous antioxidant enzymes SOD and GSH-Px in healthy subjects. Results were analysed in relation to gender, age, exercises, and smoking habit.

Methods

The study population included 257 appearently healthy individuals, 133 males (37 elderly, mean age 62 ± 12, 64 adults, mean age 38 ± 7, 32 children, mean age 12 ± 2) and 124 females (35 elderly, mean age 65 ± 11, 63 adults, mean age 36 ± 7, and 26 children, mean age 13 ± 1). Subjects were taking no medicine. We defined two age-matched groups of subjects according to their smoking habits: smokers (smoking more than 20 cigarettes per day at least 10 years) and nonsmokers. Adult individuals were stopped any physical activity for at least one week before the study and asked to run submaximal 20 minutes every day for five weeks (Aslan et al. 1998).

A total of 10 ml of blood was drawn after overnight fasting in both groups before and after the study. Of the blood sample, 5 ml of serum was collected and serum MDA levels were determined. Remaining blood was collected into tubes containing ethylenediaminotetraacetic acid (EDTA) for the preparation of erythrocytes. Blood was then centrifuged at 2000 rpm for 10 minutes in a refregerated centrifuge. Plasma and buffy coat 0.15 mol/liter NaCl solution three times. A complete blood count by electronic counter was done on washed cell samples to check for contamination by leukocytes. Preparation of washed erythr-

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ocytes and biochemical analyses were done on immediately after blood collection from individuals. Oxidative stress was estimated by the method of Jain et al. (1989) based on thiobarbituric acid (TBA) reactivity. MDA, an end product of fatty acid peroxidation, reacts with TBA to form a colored complex that has maximum absorbance at 532 nm. For this purpose, 0.2 ml serum cells were suspended in 0.8 ml phosphate-buffered saline (8.1 g NaCl, 2.302 g Na_2HPO_4 and 0.194 g NaH_2PO_4/liter, pH 7.4) and 0.025 ml butylated hydroxytoluene (88 mg/10 ml absolute alcohol). Trichloroacetic acid of 30% (0.5 ml) was added. Tubes were vortexed and allowed to stand in ice for at least 2 hours. Tubes were centrifuged at 2000 rpm for 15 minutes. One ml of each supernatant was transferred to another tube. To this, we added 0.075 ml 0.1 mol/liter EDTA and 0.25 ml 1% TBA in 0.05 mol/liter NaOH. Tubes were mixed and kept in a boiling water bath for 15 minutes. Absorbance was read at 532 nm after tubes were cooled to room temperature. BHT, an antioxidant, was added to prevent MDA formation during the assay, which could result in falsely elevated TBA reactivity. MDA values in nmol per ml serum were determined with the adsorbance coefficient of MDA-TBA complex at 532 nm $= 1.56 \times 10^5$/cm/mol.

Erythrocyte SOD and GSH-Px activities were estimated on hemolysates by use of commercial available kits (Randox lab., Dublin, Ireland). Erythrocytes were hemolysed by addition of redistilled water and vigorous vortexing. SOD estimation was based on the generation of superoxide radicals produced by xantine and xantine oxidase, which reacts with 2-(4-iodophenyl)-3-(4-nitrophenol9-5-phenyltetrazolium chloride to form a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction. GSH-Px estimation was based on the following principle: GSH-Px catalyses the oxidation of glutathion by cumen hydroperoxide. In the presence of glutathione reductase and NADPH the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured. The technique is based on the method of Paglia and Valentine (1967).

Statistical analysis was determined by Student t-test. The values were expressed as mean $\pm$ S.D.

Results

Sex and age influence

No significant change was appearent between MDA, SOD, GSH-Px levels determined in the male subjects and those in the females in blood ($p > 0.05$) (Table 1).

The mean MDA, SOD, GSH-Px levels were compared in three group without sex distinction: 9–14, 27–45, and 57–71 years. Values varied significantly with age, as shown in Table 2. Serum MDA level was higher in elderly than in children and in adults ($p < 0.05$). SOD and GSH-Px levels of children and adults were significantly higher than those of elderly group ($p < 0.05$).

Cigarette smoking influence

Serum MDA level of smokers was higher whereas SOD activity was lower than those of nonsmokers and GSH-Px didn't change significantly (Table 3).

Acute physical activity effect

Adult volunteers in the sedentary groups (Untrained individuals) run 20 minutes submaximally. MDA, SOD and GSH-Px levels were measured after 20 minutes run. There were a significant reduction in blood SOD and GSH-Px activities and an increase in MDA level (Table 4).

Regular training

Individuals exercised regularly by running 20 minutes submaximally everyday for 5 weeks training program. When subjects practiced

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TABLE 1. *The MDA, SOD, and GSH-Px concentrations according to sex*

Age	Sex	n 257	MDA nmol/ml	SOD U/gHb	GSH-Px U/gHb
9–14	Male	32	1.25±0.19	1610±590	65.12±4.09
	Female	26	1.17±0.44	1652±512	64.21±4.70
27–45	Male	64	1.72±0.14	1735±592	70.12±5.90
	Female	63	1.65±0.35	1753±452	78.24±8.10
57–71	Male	37	2.89±0.25	1105±437	58.60±4.10
	Female	35	2.79±0.21	972±348	57.48±5.92

TABLE 2. *The MDA, SOD, and GSH-Px concentrations according to age*

Age	n 257	MDA nmol/ml	SOD U/gHb	GSH-Px U/gHb
9–14	58	1.21±0.32	1631±551[b]	64.67±4.40[b]
27–45	127	1.69±0.25	1744±522[b]	74.18±7.0[b]
57–71	72	2.84±0.23[a]	1039±40	58.04±5.01

[a] $p < 0.05$ compared to children and adults.
[b] $p < 0.05$ compared to elderly.

TABLE 3. *Influence of smoking habit on MDA, SOD, and GSH-Px levels*

	MDA nmol/ml	SOD U/gHb	GSH-Px U/gHb
Smokers $n=44$ mean age $=39\pm6$	2.57±0.32	1225±3.12	65.22±15.1
Nonsmokers $n=47$ mean age $=37\pm5$	1.48±0.29*	1897±595*	73.4±5.62

* $p < 0.01$.

TABLE 4. *Effect of acute exercise on sedentary individuals (n = 32) (Adult individuals running at least 20 minutes and until achieving heart rate of 120 beats per minutes)*

	MDA nmol/ml	SOD U/gHb	GSH-Px U/gHb
Before exercise	1.37±0.09	1993±472	75.17±5.3
After acute exercise	2.14±0.21[a]	1453±695[b]	66.29±6.1[b]

[a] $p < 0.01$. [b] $p < 0.05$.

TABLE 5. *The values in trained individuals (n = 32) (Adult individuals running at least 20 minutes every day for five weeks)*

	MDA nmol/ml	SOD U/gHb	GSH-Px U/gHb
Before exercise	1.37±0.09	1993±472	75.17±5.3
After training	1.41±0.15	2067±580	79.12±5.23

$p > 0.05$.

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regular physical activity (Table 5) there was an insignificant increase of MDA, SOD and GSH-Px.

Discussion

Human studies have suggested that free radicals and radical mediated lipid peroxidation reactions and endogenous antioxidant enzymes can alter according to sex (Bast et al. 1991), age (Robertson et al. 1991), life styles (Robertson et al. 1991), environment (Pal 1994), and habits (Bridges et al. 1993). The role of free radicals and oxidant injury has been repeatedly described in various diseases but rarely healthy people so this article first examines MDA, SOD and GSH-Px concentrations on healthy human subjects and shows significance in alteration (De La Torre et al. 1999; Mecocci et al. 2000; Sekeroğlu et al. 2000). We established reference ranges of MDA, SOD and GSH-Px levels in appearently healthy subjects. An important advantage of this study when compared with those already published was the large total number of subjects ($n=257$) although the number of each compared groups is not so great. Another important point is that our samples come from a general population.

MDA, SOD, and GSH-Px levels measured in males and females are not different in our results. Regarding aging, we observed the lowest SOD and GSH-Px concentrations in elderly group, and no variation between children and adults. The same pattern has been reported by other authors (Al-Turk et al. 1987; Matsubara and Machado 1991; Lang et al. 1992). However, Inal et al. (2001) reported that although SOD activities in elderly were significantly lower than children and adult, but not the GSH-Px activities. This study, another study on turkish population, have also revealed similar results correlated with ours. Some authors, however, have observed a decreased activities of SOD and GSH-Px, in their children group (Michelet 1995). This finding against ours may be related to different population. In smokers, we observed that smoking leds to greater MDA level and lower SOD activities. Our results agree with those obtained by Bridges et al. (1993), who determined that the MDA concentration in erythrocytes from subjects smoking one pack of cigarettes or more per day was greater than non-smokers. These findings can be of interest for diseases such as chronic obstructive pulmonary diseases linked to lipid peroxidation in smoking and exposure to polluted air.

Acute bouts of exercise led to enhanced level of blood MDA and decreased levels of SOD and GSH-Px. Similar results were described by Robertson et al. (1991). These results can explain that there may be a relation between an asthmatic attack and acute exercise. However, regular participation to physical training can enhance antioxidant status (Clarkson 1995).

Finally, we concluded that there were exact relations between MDA, SOD, and GSH-Px levels and age and some life habits such as smoking, and regular or occasional physical activity in healthy subjects. We also think that blood MDA, SOD, and GSH-Px levels or one of them seem to be effected by age, smoking, and exercise but not by sex in healthy individuals.

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